CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR. — No. 144.

ON THE STRUCTURE OF THE OUTER SEGMENTS OF THE RODS IN THE RETINA OF VERTEBRATES.

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SOME forty years ago the structure of the rods in the retina of vertebrates excited a lively interest and received the attention of many skilled observers, whose researches were directed principally to the rods in the amphibians because of the advantage offered by the large size of these organs. Good summaries of these early investigations have been given by Hoffmann ('73–78), and by Krause ('92).

From the first of these sources the following description of the outer segment of the rod is taken. The outer segment is composed of a highly refractive substance staining black with osmic acid. In form it is cylindrical with a hemispherical, slightly bulging, distal end. Under high magnification its outer surface is seen to be marked by parallel striations which deviate from a strictly longitudinal course only in that they are very slightly spiral. This appearance is due to superficial furrows alternating with ridges. The form of the outer segment may thus be well compared to that of a column with a slightly spiral fluting. In addition to the longitudinal striations, transverse bands are present at regular intervals. These are surface indications of a plate structure. The plates of which the outer segment is composed are constant in thickness for a given species and show little variation even in the whole vertebrate series. They are held together by a cement lying between their approximated faces. This cement is affected rapidly by certain reagents and by its swelling causes a characteristic disintegration of the outer segment into disks. This disintegration occurs earlier at

the distal end of the segment than at the proximal one, the difference having been attributed to a protecting sheath over the latter. The presence of an axial fiber, as maintained by Ritter and others, was discredited because of much negative evidence.

In contrast to this general view Krause ('92), whose comparative studies included particularly Rana and Salamandra, maintained that the outer segment consisted of a bright "Grundsubstanz," in the periphery of which fibrils were imbedded and twisted in so close a spiral that they gave the appearance of transverse striations, thus producing a condition favorable to apparent transverse fracture. Greeff (:00, p. 103), however, has recently expressed himself in favor of the older view that the outer segment consists of a series of plates with an enveloping sheath. "Jedes Aussenglied besteht 1. Aus einer *Hülle* (Mantelschicht, Rinde, Haut) und 2. einem aus Plättchen und Zwischensubstanz gebildeten *Inhalt*." This view is also accepted by Levi (:01).

Bernard (:00, :01), who has published an account of the embryonic as well as of the adult rods in amphibians, has maintained that these structures are protrusions from a syncytial retina and that each rod is a delicate protoplasmic vesicle traversed by a reticulum which eventually becomes condensed into the axis of the rod by the absorption of a colorless refractive and amorphous matter from the pigment cells. Thus even among recent investigators much difference of opinion exists as to the structure of the vertebrate rods.

The progress made in the study of the rods in vertebrates during the last twenty-five years stands in considerable contrast with that made in the investigation of the terminal optic organs of invertebrates. In the arthropods, for instance, the rhabdomes, the analogues of the rods and cones of vertebrates, were supposed by most of the earlier writers to have been formed by secretion, and in fact Watase ('90) went so far as to compare them with surface cuticula. Their fibrous character, however, was observed by Patten ('86) and others and it was demonstrated by Parker ('95) that in the crayfish the fibrils composing them are neurofibrils, and that the substance of the rhabdome is more correctly described as differentiated living material like the

contractile substance of a muscle fibre, than as a secretion. This view that the rhabdome is composed of neurofibrils has been greatly extended among the invertebrates by the recent work of Hesse (:00, :01).

Since in some crustaceans the rhabdomes are not only fibrous but are composed of plates not unlike the so-called plates in the rods of vertebrates, it is natural to ask whether the rods in vertebrates may not also be fibrous, and with this question the present paper is chiefly concerned. The ease with which frogs could be obtained at all seasons and the comparatively large size of their rods led me to investigate the retinas of these animals and I chose in particular the common leopard frog, Rana pipiens Schreber, as a favorable species.

For a satisfactory study of the rods it was necessary to free them from their surrounding pigment. This was done in the usual way by keeping the animal two or three hours in the dark. In such "dark frogs" the retinal pigment completely withdraws from the region between the rods into the bodies of the retinal pigment cells.

Of the various methods for obtaining unwrinkled retinæ the following was used with success, and I know of no other which preserves the eye in as natural a condition as this does. Frogs kept the usual length of time in the dark were etherized, their hearts were exposed, and fixing fluid was injected into their arteries as in ordinary injections to demonstrate the arterial system. The fluids used were Vom Rath's picro-platino-osmo-acetic mixture, $\frac{1}{2}\%$ osmic acid, corrosive-acetic mixture, and Perényi's fluid.

The last two penetrated most successfully. The osmic preparations were only partially successful, for, owing apparently to the rapid constriction of the blood vessels, a smaller amount of the fluid reached the interior of the eye than by the other methods. After injection, the whole head was immersed in the fixing fluid and the eyes were not opened until they were more or less fixed. Eyes thus prepared were embedded in paraffine and cut for longitudinal or transverse sections of the rods. The sections were stained in Heidenhain's iron-hæmatoxylin, Böhmer's hæmatoxylin, Mayer's hæmacalcium, and by Kupffer's and

Bethe's methods for neurofibrils. Preparations were also made by the cover-glass method for blood technique. This is well adapted for experimenting with a large number of reagents and stains, and has the advantage of insuring immediate fixation.

The examination of material prepared in the ways enumerated gave evidence of a well marked axial core in the outer segment of each rod. This core was seen in both longitudinal and transverse sections of rods fixed in the various fluids already mentioned and measured about one fourth the diameter of the rod. It took none of the stains which I have tried with the possible exception of picric acid. It is probably the structure long ago seen by Dreser ('86) and recently identified by Bernard (: 01, p. 465) as the condensed reticular portion in the axis of the rod. Its relative thickness precludes the possibility of its being the so-called fibre of Ritter, if in fact this fibre exists. At present I do not wish to express any opinion as to the exact nature of this core.

As previously stated the substance of the rods has been variously described as lamellar, spirally fibrous, etc. Since rods prepared by different methods showed much difference in structure, it was necessary to study fresh ones as a means of interpreting what was seen in the preserved preparations. But under the ordinary microscope the substance of fresh rods appeared to be almost homogeneous and I was, therefore, obliged to seek other means of studying these bodies. The problem thus resolved itself into a search for conditions which would bring out optical differentiation in an object which under ordinary circumstances seemed optically homogeneous. Polarized light seemed the most likely means, for, if the rods are fibrous not only ought this to be open to determination by a polarizing microscope, but it ought also to be possible by the same means to ascertain the direction of the fibrils.

A polarizing microscope was used with a powerful artificial white light and a gypsum interference plate inserted between the Nicol prisms. The prisms were placed at such an angle to each other as to give an interference color of a sensitive violet of the first order. With the apparatus thus set up fresh preparations of the retina more or less teased out were examined.

In such preparations fields may easily be found containing detached rods with their longitudinal axes lying in various directions.

Outer segments lying parallel to the *a* axis of the gypsum plate, $\pm 45°$ to the cross-hairs, showed a bright yellow color, while those at right angles to this were bright blue. The colors of an individual rod could be reversed by turning the preparation so as to bring the rod into a line at right angles to its former position. The inner segments of the rods are not highly refractive. These observations were made on the rods of Rana pipiens but I have also tested the outer segments of the rods or cones, as the case may be, in the mudpuppy (Necturus), turtle, snake, lizard (Anolis), guinea pig, mouse, and ox, and with wholly confirmatory results.

This definite reaction demonstrates that the substance of the outer segments is positively doubly refractive or anisotropic, *i. e.*, as regards their optical properties the outer segments have axes of maximum elasticity at right angles to their lengths. To obtain an immediate basis for comparison I made similar tests of other tissues. Thus bundles of naked axis cylinders from the inner surface of the vertebrate retina gave light reactions exactly like those given by the outer segments of the rods and the same was true of striped muscle fibres from the crayfish, frog, and ox as well as of connective tissue fibres from the ligamentum nuchæ. The rhabdomes from the compound eye of the crayfish were, however, negatively anisotropic, but when it is remembered that the fibrous structure of these bodies is at right angles to their length instead of being parallel to it as in all the other bodies tested, this apparent exception disappears. Since the neurofibrils are known to run lengthwise the axis-cylinders of nerves and since naked axis-cylinders and the outer segments of the rods give the same color reactions in the polarizing microscope, I believe I am justified in concluding that fresh outer segments of the rods of vertebrates like axis cylinders of nerve fibres possess a longitudinal fibrillation.

The color reactions just recorded are directly opposed to Krause's conception of the rods as made up of spirally twisted fibrils. Such a structure would give color reaction the opposite

to those actually seen, for the fibrils would be nearly at right angles to the longitudinal axis of the rod. Nor do these reactions favor the view held by Bernard (:01) that the rods are protoplasmic vesicles filled with an amorphous refractive substance, for the material is not amorphous but gives evidence of longitudinal fibrillation. Patten's ('98) hypothesis that the outer segments are made up of minute fibrils at right angles to their longitudinal axes is also inconsistent with these observations.

Although the evidence I have advanced cannot be said to be opposed to the generally accepted view that the rod is made up of many disk-shaped plates, I am not inclined to place so much emphasis on this as some have done. I have obtained abundant evidence for the presence in fresh rods of transverse bands about equal to each other in thickness and held together by an intermediate substance of different optical behavior. But I have not found the evidence for the disintegration of a rod into disks at all convincing. There were certainly frequent instances of transverse breaking, but it was seldom clear cut and there were often signs of longitudinal splitting and of spreading at broken ends.

I believe we have in the rod certain conditions analogous to those of striped muscle fibres. Both bodies are positively refractive, both possess a transverse lamellar arrangement of optically differing substances, and under the action of certain reagents both are said to break into transverse segments.

The structure of the muscle fibre is essentially fibrillar notwithstanding its transverse fracture and I believe the structure of the outer segment of the retinal rod to be in this respect like that of the muscle fibre.

Having given the evidence for the longitudinal fibrous structure of the rods as I have found it by the use of polarized light, I wish to discuss some contradictory results already recorded as having been obtained by this method. Valentin ('62) investigated with polarized light a large number of animal tissues including the rods of the retina and the axis cylinders of nerves, and, as the following quotations show, he found that the reactions of these two bodies were not similar but opposite. "Die nähere Verfolgung des Gegenstandes zeigt, das die optische Axe der Längsaxe der Nerven parallel geht, man also hier

einen wahrhaft negativen Körper vor sich hat und die ganze Erscheinung nur von dem Marke herrührt" (Valentin, '62, p. 123). "Man könnte theoretisch annehmen, das die Stäbchen an und für sich nicht anders, als die markigen Nervenfasern wirken" (p. 136). "Jene (Stäbchen) wären aber wahrhaft positiv und das (Nerven) Mark von diesen wahrhaft negativ" (p. 136).

It is thus evident that Valentin believed that the optical axes of the rods and of the nerve fibres were not in agreement but were at right angles to each other, and this opinion was accepted by Max Schultze ('67), Krause ('92),[1] and Greeff (:00).

It is not easy to account for Valentin's statement that the axis cylinders of nerves are negatively anisotropic unless we assume that in consequence of the imperfect knowledge of nerve structure at his time he has recorded the reaction of the medullary sheath, which is negative, instead of that of the axis cylinder. Valentin's work was done on Torpedo marmorata and shows that his observations were made almost entirely upon medullated nerves. It is quite evident that what he refers to as sheaths of the nerve must have been the positively reacting connective tissue of the peripheral nerves, for he makes no mention whatever of the brilliantly conspicuous medullary sheath as such. He does, however, speak of pressing out the retina of a frog with a cover-glass and finding fibres which he considers to be parts of the optic nerve. These, he states, also showed negative reactions, but there is no certainty that what he described were really optic nerve fibres.

In my tests of nerves I found medullated fibres unsatisfactory objects for clear demonstration of optical properties in the axis cylinder, because of the strong predominance of the reaction color of the medullary sheath. The non-medullated fibres from invertebrates (crayfish) were more satisfactory, but even here the presence of the positive Schwann's sheath, though comparatively thin, made conclusive observation out of the question for the color of the sheath was projected on the less strongly reacting axis.

[1] "Die Aussenglieder sind ferner positiv doppelbrechend, die optische Axe liegt in ihrer Längsrichtung und es ist bemerkenswert das sie sich entgegengesetzt wie das bekanntlich negativ Nervenmark verhalten." (Krause, '92, p. 159.)

It was, therefore, necessary to use nerve fibres without protective coverings. The naked axis cylinders radiating from the entering optic nerve in the fibre layer of the retina, met this requirement. In order to get a clear demonstration of these, I made tests upon the retina from a perfectly fresh ox eye where the large size of the eye made manipulation comparatively simple. In this case there was little difficulty in identifying the radiating bundles of nerve fibres which were readily distinguishable from small blood vessels and other structures of a fibrous nature. The bundles of naked axis cylinders proved to be distinctly *positive*, thus agreeing with the rods and I am consequently forced to conclude that in some way Valentin's observations were in this respect erroneous.

Summary. The outer segments of the rods in the retina of the frog contain each an axial core that differs from the peripheral substance, but the exact nature of this core has not yet been made out. The outer segments, as demonstrated by the use of polarized light, are positively anisotropic and agree in this respect with the axis cylinders of nerves. These outer segments therefore, give evidence of containing longitudinal fibrillæ. Since they also show in the fresh state a transverse banding, their structure is in some respects not unlike that of a cross-striped muscle fibre in that in addition to a cross banding they also possess a longitudinal fibrillation.

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